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EXAMINER

NGUYEN, QUANG

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Please find below and/or attached an Office communication concerning this application or proceeding.

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**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Application Number: 10/082,772
Filing Date: February 25, 2002
Appellant(s): DROGE ET AL.

Steven L. Highlander, Reg. No. 37,642
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 5/21/08 appealing from the Office action mailed 2/21/08.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

No amendment after final has been filed.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

GROUND OF REJECTION NOT ON REVIEW

The following grounds of rejection have not been withdrawn by the examiner, but they are not under review on appeal because they have not been presented for review in the appellant's brief. Claims 29 and 49-51 are rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. (US 6,143,530) in view of Calos (US 6,632,672), Hartley et al. (US 5,888,732) and Christ & Droge (J. Mol. Biol. 288:825-836, 1999).

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

6,143,530	Crouzet et al.
5,464,764	Capecchi et al.
5,888,732	Hartley et al.

Lange-Gustafson et al. "Purification and properties of Int-h, a variant protein involved in site-specific recombination of bacteriophage lambda". J. Biol. Chem., vol259, no. 20 (October 25, 1984), pp. 12724-12732.

Christ et al. "Alterations in the directionality of lambda site-specific recombination catalyzed by mutant integrases in vivo". J. Mol. Biol., vol288 (May 21, 1998), pp. 825-836.

Declaration of Peter Droge under 37 C.F.R. 1.132, filed on 10/04/06.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

(a) *Claims 29-30, 32-33, 36, 38, 44-48 and 58 are rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. (US Patent 6,143,530) in view of Christ & Droge (J. Mol. Biol. 288:825-836, 1999).*

Crouzet et al teach a method for producing therapeutic DNA molecules by excision from a plasmid or from a chromosome (must be stably integrated) by site-specific recombination, wherein the site-specific recombination is carried out in a host cell or *in vitro*, and wherein the site-specific recombination is carried out by means of various systems which lead to site-specific recombination between sequences (col. 3, line 27 continues to line 20 of col. 4; Figures 1-2). Specifically, Crouzet et al disclose that the gene or genes of interest flanked by the two sequences permitting site-specific recombination, positioned in the direct orientation in a vector, a phage or a virus, and the specific recombination sequences and recombinases used can belong to different structural classes, including the integrase family of bacteriophage λ and the attP and attB sequences catalyzed by the phage lambda integrase (col. 4, lines 21-52; col. 5, lines 32-41). The disclosed attB sequence is identical to SEQ ID NO:1 of the present application (see SEQ ID NO:6), and therefore the corresponding attP recombination sequence must have the same SEQ ID NO: 2 of the present application, or at least a derivative thereof. Additionally, Crouzet et al. teach that their genetic constructs can be used for the construct of cell hosts having disclosed elements integrated in their genomes (col. 4, lines 4-9; col. 8, lines 64; col. 9, lines 27-60; and Figure 1).

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Crouzet et al further teach that the recombinase to be introduced into a host cell can be under the control of a promoter or a system of inducible promoters, and it can be carried by a plasmid, a phage or even in the non-therapeutic region of the recombinant DNAs (col. 7, line 45 continues to line 39 of col. 8), and that any type of host cell can be used including eukaryotic cells such as yeasts, animal cells, plant cells, mammalian cells such as CHO, COS and mouse NIH3T3 (col. 9, lines 48-60).

Crouzet et al also disclose that their genetic constructs comprise one or more genes of interest such as the genes coding for enzymes, hormones, growth factors, apolipoproteins, tumor suppressor genes, suicide genes, natural or artificial immunoglobulin or an antigenic peptide (col. 13, lines 14-50).

Crouzet et al do not specifically teach the use of any modified λ Integrase, specifically Int-h or Int h/218 in their method of producing therapeutic DNA molecules, even though they teach that any specific recombination sequences and appropriate recombinases can be used, including those belonging to the bacteriophage λ system.

However, at the effective filing date of the present application Christ & Droge already taught the mutant λ Integrases, Int-h and Int-h/218, that are capable of mediating substrate deletion through λ site-specific recombination in prokaryotes in the absence of co-factors Fis and Xis (see at least the abstract; page 829, right hand column, top and bottom of the first full paragraph). Christ & Droge further teach that the potential of these mutants to perform recombination in the absence of accessory factors is due to their enhanced affinity for core sites, and that Int-h is known to recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more

efficiently than wild-type Integrase (page 827, left column, second full paragraph; page 828, left column, top of last paragraph).

Accordingly, it would have been obvious for an ordinary skilled artisan to modify the method taught by Crouzet et al by utilizing specifically the mutant λ Integrases, Int-h and Int-h/218, of Christ & Droge in their method of producing therapeutic DNA molecules due to the advantages offered by the mutant integrases, at least the mutant Integrases can recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more efficiently than the wild-type Intergrase.

An ordinary skilled artisan would have been motivated to carry out the above modification because as taught by Christ & Droge, at least these mutant Integrases can recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more efficiently than wild-type Integrase.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Crouzet et al., Christ & Droge, coupled with a high level of skill of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

(b) *Amended claims 29 and 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. (US Patent 6,143,530) in view of Christ & Droge (J.*

Mol. Biol. 288:825-836, 1999) as applied to claims 29-30, 32-33, 36, 38, 44-48 and 58 above, and further in view of *Capecchi et al.* (US 5,464,764).

The teachings of Crouzet et al. and Christ & Droge have been discussed above. However, neither Crouzet et al nor Christ & Droge teach that the first and/or the second DNA segment further comprising a sequence effecting integration of said first and/or second DNA segment into the genome of a cell by homologous recombination, even though Crouzet et al teach specifically that the genetic constructs can be used for the construct of cell hosts having disclosed elements integrated in their genomes and that the insertion may also be carried out by homologous recombination (col. 4, lines 4-9; col. 8, lines 60-64; and Figure 1).

However, at the effective filing date of the present application *Capecchi et al.* already describe a well-known method and a positive-negative selector vectors comprising sequences to be introduced in the genome of a target cell by the homologous recombination approach (see abstract and Figure 1).

It would have been obvious for an ordinary skilled artisan to further modify the modified method of Crouzet et al and Christ & Droge by introducing the genetic construct in the form of a positive-negative selector vector described by *Capecchi et al.* into a target host cell capable of homologous recombination to incorporate their genetic construct in the genome of the host cell as specifically taught by Crouzet et al.

An ordinary skilled artisan would have been motivated to carry out the above modification because the approach taught by *Capecchi et al.* overcomes many

problems associated with random integration, and that the gene of interest can be introduced into any predetermined region of the genome of a target host cell.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Crouzet et al., Christ & Droge and Capecchi et al, coupled with a high level of skill of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

(c) *Amended claims 29, 34-35, 36-37 and 39 are rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. (US Patent 6,143,530) in view of Christ & Droge (J. Mol. Biol. 288:825-836, 1999) as applied to claims 29-30, 32-33, 36, 38, 44-48 and 58 above, and further in view of Hartley et al. (US 5,888,732).*

The teachings of Crouzet et al. and Christ & Droge have been discussed above. However, neither Crouzet et al nor Christ & Droge teach that the first DNA segment stably integrated into the genome of a eukaryotic cell comprising an attL sequence according to SEQ ID NO:3 or a derivative thereof, or an attR sequence according to SEQ ID NO:4 or a derivative thereof, even though Crouzet et al teach specifically that a method for producing therapeutic DNA molecules by excision from a plasmid or from a chromosome (must be stably integrated) by site-specific recombination, wherein the site-specific recombination is carried out in a host cell or *in vitro*, and wherein the site-specific recombination is carried out by means of various systems which lead to site-

specific recombination between sequences, including the use of bacteriophage λ and the attP and attB sequences (col. 3, line 27 continues to line 20 of col. 4; Figures 1-2).

However, at the effective filing date of the present application Hartley et al already taught both *in vitro* and *in vivo* (e.g., in eukaryotic host cells) methods for the exchange of DNA segments through the use of various recombination proteins described in the art, including λ Integrase (see at least Summary of the Invention; col. 13, lines 35-55 and line 57 continues to line 24 of col. 16) and its recognition sequences including attB, attP, attL, and attR sequences (col. 8, lines 43-63). The disclosed attL and attR recombination sequences that are catalyzed by λ Integrase taught by Hartley et al. must have the same SEQ ID NO:3 and SEQ ID NO:4, respectively, or at least a derivative thereof. Hartley et al. also teach the use of integrase in the presence of λ protein Xis (excise) to catalyze the reaction of attR and attL (col. 15, lines 1-3). Hartley et al. also teach engineered att recombination sites having one or multiple mutations to enhance specificity or efficiency of the recombination reaction, and that a tripartite protein fusion could be accomplished with parental plasmids containing recombination sites attR1 and attR2; attL1 and attL3; and attR3 and attL2 (col. 16, lines 26-61; col. 17, line 47 continues to line 46 of col. 18).

It would have been obvious for an ordinary skilled artisan to modify the method of Crouzet et al and Christ & Droge by also utilizing the λ Integrase recognition sequences attL and attR sequences flanking the gene or genes of interest, optionally along with a source of Xis factor under the control of a promoter or a system of inducible promoters, for the production of therapeutic DNA molecules. Once again, it is noted that Crouzet et

al teach specifically that the site-specific recombination is carried by means of various systems which lead to site-specific recombination between sequences.

An ordinary skilled artisan would have been motivated to carry out the above modification because the excision system involving λ Integrase and its recognition sites attL and attR sequences is well known and already taught by Hartley et al for the exchange of DNA segment in both *in vitro* and *in vivo*.

An ordinary skilled artisan would have a reasonable expectation of success in light of the teachings of Crouzet et al., Christ & Droge and Hartley et al, coupled with a high level of skill of an ordinary skilled artisan in the relevant art.

Therefore, the claimed invention as a whole was *prima facie* obvious in the absence of evidence to the contrary.

(10) Response to Argument

(a) *Response to arguments for the rejection of claims 29-30, 32-33, 36, 38, 44-48 and 58 are rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. (US Patent 6,143,530) in view of Christ & Droge (J. Mol. Biol. 288:825-836, 1999).*

1. Appellant argues that Crouzet et al worked with wild-type integrases in eukaryotic cells, while Christ & Droge worked in prokaryotic systems with mutant intergrases; and it is Appellant's position that there was no a priori expectation of success even if motivation for combining these two very distinct systems were presumed. Appellant also argue that the examiner's argument rests solely upon the

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alleged statement that "the conditions required by wild-type lambda integrase to mediate recombination actions in prokaryotic cells, under physiological conditions and in vitro conditions are apparently more stringent than those required by Int-h...", and that this evidence is supposed supported by Hartley et al, Christ & Droge and Lange-Gustafson et al. With respect to the Hartley et al reference, Appellant argues that this reference makes no mention of modified integrases and therefore it is impossible for Hartley to provide any comparative statement about the stringency of a modified int's activity relative to that of wild-type lambda integrases. With respect to the Lange-Gustafson et al reference, Appellant argues that considering the data of Lange-Gustafson that allegedly show that Int-h works better with supercoiled DNA, it undercuts the relevance of this reference. Additionally, Appellants argues that the studies in the reference were performed in vitro and reflect conditions that have nothing to do with the environment inside a living eukaryotic cell. With respect to the Christ & Droge reference, Appellant argues that the work reported in this reference on modified integrases was all performed in prokaryotic cells and whatever the requirements observed in such prokaryotic cells can not be extrapolated to the activity in eukaryotic cells.

With respect to the main issue whether the modified integrases Int-h and Int-h/218 would work in eukaryotic cells, based on the teachings of cited prior art of Hartley et al, Christ & Droge and Lange-Gustafson et al an ordinary skilled artisan would reasonably expect at least Int-h or both Int-h and Int-h/218 to function in eukaryotic cells for the following reasons:

(i) Although the Hartley et al reference does not mention Int-h or Int^h/218, the reference teaches clearly that wild-type λ integrase is capable of mediating the exchange of DNA fragments in both *in vitro* and *in vivo* in cells including both eukaryotic and prokaryotic cells (col. 13, lines 35-55), regardless whether the DNA is negative supercoiled (prokaryotic cell genome) or relaxed (eukaryotic genome). Moreover, Hartley et al disclose specifically that wild-type λ Integrase requires accessory factors such as IHF protein for integrative recombination and IHF and Xis proteins for excisive recombination (col. 14, line 26 continues to line 4 of col. 15). It should be noted the same basic teachings are taught in the primary reference of Crouzet et al.

(ii) Lange-Gustafson et al already stated "In contrast to the wild-type int gene product (Int⁺), which produces almost no recombinants in the absence of IHF, purified Int-h protein sponsors reduced but significant levels of integrative recombination in the absence of any *E. Coli* supplement. This shows that the int gene encodes all the information necessary for the elementary steps in recombination and implies that IHF functions as an accessory protein" (first paragraph of the abstract); "When supplemented by IHF, recombination promoted by Int-h resembles that promoted by Int⁺ in kinetics, stoichiometry of Int and IHF, and nature of the recombinant product. Under these conditions, Int-h uses supercoiled DNA more effectively than nonsupercoiled DNA as a substrate for recombination, as does Int⁺. However, in the absence of IHF, Int-h recombines supercoiled and nonsupercoiled substrates identifiably, indicating that IHF is an

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important part of the mechanism that senses the supercoiled state of the substrate DNA during recombination" (top of second paragraph of the abstract). These statements do not undercut the examiner's position in any shape or form as argued by Appellant. Only when supplemented by IHF, Int-h uses supercoiled DNA more effectively than nonsupercoiled DNA. **This does not mean that under this specific condition, Int-h would not mediate recombination with nonsupercoiled DNA.** Moreover, Lange-Gustafson et al also disclose specifically that **in the absence of IHF, Int-h recombines supercoiled and nonsupercoiled substrates identically. Thus, the recombination activity of Int-h is not dependent on the supercoiled and/or relaxed state of a DNA substrate.** This is also supported by the statement "**the int gene encodes all the information necessary for the elementary steps in recombination**".

(iii) Although Christ & Droge did not perform site-specific recombination in eukaryotic cells, they teach specifically that the mutant λ Integrases, Int-h and Int-h/218, are capable of mediating substrate deletion through λ site-specific recombination in prokaryotes **in the absence of co-factors Fis and Xis** (see at least the abstract; page 829, right hand column, top and bottom of the first full paragraph). Christ & Droge further teach that **the potential of these mutants to perform recombination in the absence of accessory factors is due to their enhanced affinity for core sites, and that Int-h is known to recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more efficiently than wild-type Integrase** (page 827, left column, second full paragraph; page 828, left column, top of last paragraph). Moreover, Table 1 also indicates clearly in contrast to Int-h or Int-h/218,

wild-type Int usually requires an accessory IHF for inversion and/or deletion reactions.

Accordingly, based on the analysis of the state of the prior art at least through the teachings of Crouzet et al., Hartley et al., Lange-Gustafson et al., and Christ & Droge as discussed above, **an ordinary skilled artisan would reasonably expect at least Int-h or both Int-h and Int-h/218 to function in eukaryotic cells. It is also abundantly clear that the conditions that are required by a wild-type λ integrase to mediate recombination in prokaryotic cells, in vitro conditions and in vivo are more stringent than those required by the Int-h or Int-h/218 (without the requirement of accessory factors).** With respect to the motivation for combining the teachings of Crouzet et al and Christ & Droge, as already noted in the above rejection, an ordinary skilled artisan would have been motivated to modify the method taught by Crouzet et al by utilizing specifically the mutant λ Integrases, Int-h and Int-h/218, of Christ & Droge in their method of producing therapeutic DNA molecules due to the advantages offered by the mutant integrases, at least the mutant Integrases can recombine pairs of att sites which differ in their overlap sequence by one or more base-pairs more efficiently than the wild-type Integrase.

2. Appellant further argues that the examiner has dismissed evidence provided by Appellant in the form of the Rule 132 declaration (Exhibit 6). In the Declaration, Dr. Droge explained that **the skilled artisan could not predict the success of using modified integrases in eukaryotic cells for the simple reason that it is well known that the organization of the prokaryotic genome is distinct**

from eukaryotics. In the Declaration, Appellant stated **"However, one has to realize that DNA substrates (whether episomal or genomic) are negatively supercoiled inside *E. coli*. It was, therefore, not obvious to one of ordinary skill to deduce from the existing data that the mutant recombinase would work inside mammalian cells where the DNA is topologically relaxed"** (page 2, lines 9-12 of paragraph numbered 3). In this Appeal Brief, Appellant stated **"Without the aid of topologically underwound DNA, which exists only in prokaryotic cells, it was reasonable to assume that mutant Int proteins cannot function"** (page 7, lines 4-5). Appellant further argues that the only fact of record supports appellant's position regarding unpredictability and that alone is sufficient to undercut the examiner's position. The basis premise for the rejection that one can assume that modified integrase will operate in eukaryotic cells based on their performance in prokaryotic cells is flawed as a matter of scientific principle because it could very well have been the case that what made them allegedly work "better" in prokaryotic cells, as compared to wild-type integrase, would have worked against them in the context of eukaryotic cells.

(i) The Examiner has considered fully the Declaration filed under 37 CFR 1.132, filed on 10/04/06 but it was not found persuasive to overcome the rejection of record. Initially, the Examiner would like to note that the cited paragraphs in the Declaration and in this Appeal Brief appear to be contradictory. While the Declaration states that the **DNA substrates are negatively supercoiled inside *E. coli* while the DNA inside mammalian cells is topologically relaxed**, Appellant states in this Appeal Brief that **without the aid of tologically underwound DNA (implying relaxed DNA?)**.

which exists only in prokaryotic cells, it was reasonable to assume that mutant Int proteins can not function.

(ii) Nevertheless, since wild-type lambda integrases are capable of mediating sequence specific recombination events in both eukaryotic (DNA substrates are topologically relaxed) and prokaryotic cells (DNA substrates are negatively supercoiled), and it is known in the prior art that the conditions required by a wild-type lambda integrase to mediate recombination reactions in prokaryotic cells, under physiological conditions and *in vitro* conditions are apparently more stringent than those required by the Int-h as evidenced at least by the teachings of Hartley et al., Christ & Droge and Lange-Gustafson et al as elaborated in more details above, it is therefore entirely reasonable for an ordinary skilled artisan **to expect that at least Int-h or both Int-h and Int-h/218 are able to function in eukaryotic cells**. Particularly, Lange-Gustafson et al already disclosed specifically that **in the absence of IHF, Int-h recombines supercoiled and nonsupercoiled substrates identically; and that the recombination activity of Int-h is not dependent on the supercoiled and/or relaxed state of a DNA substrate (activities under conditions with/without the presence of IHF). Appellant's arguments relating the unpredictability are not consistent with the state and the teachings of the prior art at least reflected through the teachings of Crouzet et al., Hartley et al., Lange-Gustafson et al., and Christ & Droge as discussed above.**

(iii) It also appears that Appellant requires the Examiner to support his position or conclusions with a prior art that teaches explicitly the use of Int-h or Int-h/218

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to carry out sequence specific recombination of DNA in a eukaryotic cell. However, it is noted that if such a reference exists, the reference would be qualified and used for a rejection under 35 U.S.C 102.

(b) Response to arguments for the rejection of claims 29 and 43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. (US Patent 6,143,530) in view of Christ & Droge (J. Mol. Biol. 288:825-836, 1999) as applied to claims 29-30, 32-33, 36, 38, 44-48 and 58 above, and further in view of Capecchi et al. (US 5,464,764).

Appellant argues basically that Capecchi et al reference fails to address the issue whether modified integrases would work in eukaryotic cells as already set forth above. Appellant also argues that there was no motivation for combining the primary and secondary references, and even if there were, there was no likelihood of success that they would work together.

Please refer to the same Examiner's response to Appellant' arguments on the lack of motivation and the lack of an expectation of success for combining the Crouzet et al. and Christ & Droge references in the rejection of claims 29-30, 32-33, 36, 38, 44-48 and 58 above. The teachings of Capecchi et al were used to supplement the combined teachings of Crouzet et al. and Christ & Droge on the limitation recited in dependent claim 43.

(c) *Response to arguments for the rejection of claims 29, 34-35, 36-37 and 39 are rejected under 35 U.S.C. 103(a) as being unpatentable over Crouzet et al. (US Patent 6,143,530) in view of Christ & Droge (J. Mol. Biol. 288:825-836, 1999) as applied to claims 29-30, 32-33, 36, 38, 44-48 and 58 above, and further in view of Hartley et al. (US 5,888,732).*

Once again, Appellant argues basically that Hartley et al reference fails to address the issue whether modified integrases would work in eukaryotic cells as already set forth above because the reference teaches recombinational methods in prokaryotic and eukaryotic cells using exclusively the wild-type lambda integrase; and fails to address the issue whether modified integrases would work in eukaryotic cells. Appellant also argues that there was no motivation for combining the primary and secondary references, and even if there were, there was no likelihood of success that they would work together.

Please refer to the same Examiner's response to Appellant' arguments on the lack of motivation and the lack of an expectation of success for combining the Crouzet et al. and Christ & Droge references in the rejection of claims 29-30, 32-33, 36, 38, 44-48 and 58 above. The teachings of Hartley et al were used primarily to supplement the combined teachings of Crouzet et al. and Christ & Droge on limitations recited in dependent claims 34-35, 37 and 39.

(11) Related Proceeding(s) Appendix

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No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

/QUANG NGUYEN, Ph.D./
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Conferees:

/Joseph T. Woitach/

Supervisory Patent Examiner, Art Unit 1633

/Ram R. Shukla/

Supervisory Patent Examiner, Art Unit 1634